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Analytical characteristics of a biomarker-based risk assessment test for acute kidney injury (AKI)

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ABSTRACT

Background: Acute kidney injury (AKI) is associated with increased mortality, morbidity, hospital length of stay, and costs. A quantitative urine test is available to assess the risk of developing AKI by measuring the concentrations of two protein biomarkers, TIMP-2 and IGFBP-7. The NephroCheck Test combines these concentrations into an AKIRisk Score. The purpose of this study is to characterize the analytical performance characteristics of the AKIRisk Score.

Methods: Linearity and analytical sensitivity were evaluated by following Clinical Laboratory Standards Institute (CLSI) EP06-A and EP17-A, respectively. Precision was evaluated by testing clinical samples and examining the repeatability of test results. Potential interference was evaluated for endogenous and exogenous substances. Sample stability was examined at room temperature and at 2–8 °C, as well as the effect of sample centrifugation temperature on test results.

Results: The AKIRisk Score exhibits approximately 10% coefficient of variation (CV) at the recommended cutoff value of 0.3 and the limit of quantitation (LoQ) was 0.002. Only albumin, bilirubin (conjugated), and methylene blue interfered with test results, at concentrations exceeding 1250 mg/L, 72 mg/L, and 0.49 mg/L, respectively. AKIRisk Score results were stable for 6 h at room temperature, 24 h refrigerated, and not impacted by sample centrifugation temperature.

Conclusions: Our studies demonstrate that the AKIRisk Score has robust analytical performance, good precision, minimal analytical interference, acceptable sensitivity, and excellent sample stability.

1. Introduction

Acute kidney injury (AKI) is a serious clinical condition that occurs frequently without overt warning signs or symptoms, and manifests clinically as an abrupt decrease in kidney function. AKI is often an undetected, untreated, silent killer, especially in critically ill hospitalized patients. Of the 5 million patients admitted to intensive care units (ICUs) in the United States each year, up to 50% will develop some form of AKI [1], and the most deadly forms of AKI (moderate–severe, stage 2–3) can occur in 20% or more of critically ill patients [2,3]. Short-term and long-term consequences, such as length of stay (LOS), hospital costs, 30-day readmission, and hospital mortality can be twice as severe in patients with moderate–severe, stage 2–3 AKI [4].

In terms of incidence, AKI is as common as myocardial infarction [5], and studies suggest that in hospitalized patients AKI may be twice as deadly with death rates more than breast cancer, prostate cancer, diabetes and heart failure, combined [6]. The healthcare burden for AKI is staggering and underappreciated. In the United States, the annual economic burden to the healthcare system for hospital-acquired AKI is estimated to exceed $10 billion, placing it among those complications associated with the highest in-hospital costs [7].

Until now, there have been very few options available to clinicians for identifying patients at the highest risk for moderate to severe AKI [5] and triaging vulnerable patients to potentially mitigate the consequences of AKI. Recently, the Food and Drug Administration (FDA) cleared the NephroCheck Test, a laboratory performed urine test to assess the risk of developing AKI. The NephroCheck Test quantifies concentrations of tissue inhibitor of metalloproteinase 2 (TIMP-2) and insulin-like growth factor binding protein 7 (IGFBP-7) in urine specimens. TIMP-2 and IGFPB-7 are soluble proteins expressed in the kidney that can elevate renal tubule cell stress during the earliest, potentially reversible, phases of injury. These two biomarkers are known to be involved in the response to various tissue insults such as inflammation,

Abbreviations: AKI, acute kidney injury; TIMP-2, tissue inhibitor of metalloproteinase 2; IGFBP-7, insulin-like growth factor binding protein 7; KDIGO, kidney disease improving global outcomes.

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oxidative stress, and exposure to drugs and toxins that can cause AKI. The two biomarkers are believed to invoke a protective mechanism known as G1 cell-cycle arrest [8,9]. Elevation of these two biomarkers may therefore be a signal of the kidney's attempt to protect itself when exposed to harmful insults, thus providing a "renal alarm" that heralds a state of high risk for imminent AKI [9].

The NephroCheck Test multiplies the concentrations of TIMP-2 and IGFPB-7, [TIMP-2]·[IGFBP-7] / 1000 (units = $(ng/ml)^2$ / 1000), to generate a quantitative AKI risk index (AKIRisk Score). Previous studies have shown that in apparently healthy individuals the AKIRisk Score has a normal reference range of 0.04 to 2.25 [10]. In hospitalized critically ill patients, an AKIRisk Score result greater than 0.3 is associated with seven times the risk of developing moderate to severe AKI within 12 h of assessment, compared with patients with a less than 0.3 AKIRisk Score [11]. The AKIRisk Score was initially developed in a discovery study that analyzed more than 1200 diverse ICU patients from 37 clinical sites in North America and Europe [8]. These two urinary biomarkers were subsequently validated in prospective clinical trials of more than 500 diverse ICU patients from 29 clinical sites in the United States [11,12]. Because the NephroCheck Test is the first of its kind for risk assessment of AKI, there is no predicate test, and the AKIRisk Score was therefore validated by comparison to a clinical endpoint rather than in a method comparison. The primary endpoint was moderate–severe (stage 2–3) AKI within the 12 h after sample collection as determined by clinical adjudication among a committee of three nephrologists based on Kidney Disease Improving Global Outcomes (KDIGO) Consensus criteria [13]. We report here, for the first time, the analytical performance characteristics of the NephroCheck Test and AKIRisk Score. We specifically examined the analytical linearity, precision, sensitivity, and robustness of the AKIRisk Score to potentially interfering substances, as well as sample handling and storage conditions.

2. Materials and methods

2.1. Test method

The NephroCheck Test quantitatively measures TIMP-2 and protein IGFBP-7 concentrations in adult, human urine in approximately 20 min using fluorescence, lateral flow immunoassay technology [14]. The test contains sandwich immunoassays for TIMP-2 and IGFBP-7 in a single-use plastic test cartridge. Urine samples are centrifuged, added to a buffer, and mixed with a fluorescent antibody conjugate prior to measurement.

The sample is then applied to the cartridge and inserted into a bench-top instrument that reads the fluorescent signals from each of the TIMP-2 and IGFBP-7 immunoassays. The test system displays the immunoassay results as a single numerical result called the AKIRisk Score. The AKIRisk Score result is calculated from the multiplicative product of the two biomarker results divided by 1000 (i.e.[TIMP-2]·[IGFBP-7]/1000) [14]. The AKIRisk Score has units of $(ng/ml)^2$ /1000, but is presented as a dimensionless result by the test system, and as such, the AKIRisk Score was analyzed and is reported without units in our studies.

2.2. Analytical sensitivity

The limit-of-blank (LoB), limit-of-detection (LoD), and limit-of-quantitation (LoQ) for the TIMP-2, IGFBP-7, and AKIRisk Score results were determined following CLSI guideline EP17-A [15]. A blank urine sample devoid of TIMP-2 and IGFBP-7 was tested with 72 individual NephroCheck Tests. The LoB was given by the 95th percentile of the blank sample results. LoD and LoQ were evaluated by separately testing 6 urine samples, containing low concentrations of TIMP-2 and IGFBP-7, with 36 individual NephroCheck Tests per sample across 3 days of testing, 2 test runs per day, 6 replicates per test run. The LoD was determined from the lowest concentration urine sample that exhibited less than 5% chance of falsely concluding that no analyte was present in the sample. The LoQ was determined from the lowest concentration urine sample that exhibited assay results with less than 20% total error in the TIMP-2 and IGFBP-7 assay results.

2.3. Precision study

Precision of the AKIRisk Score was evaluated by testing 1123 urine specimens collected from critically ill hospitalized patients at risk for AKI. These samples were previously collected as part of the Topaz multicenter study and followed the Helsinki Declaration of human rights [11]. The study was approved and participants provided written informed consent. Each sample was tested in singlicate across different instruments, operators, and testing runs at three different laboratories to collect a total of 3369 data points. Outliers, representing 1.2% of the data or 39 data points, were removed from this dataset according to Healy's test for outliers [16]. The remaining data were used to construct a precision profile according to previously published methods [17]. Briefly, the average AKIRisk Score result for each sample was calculated among the three testing laboratories. The results for all samples were then sorted by these average values in ascending order and grouped into bins of 20 to 50 samples. The CV and average AKIRisk Score within each bin were calculated and plotted against one another to examine the AKIRisk Score's CV as a function of its reported value.

2.4. Linearity studies

The linearity of the TIMP-2 and IGFBP-7 assays was evaluated following CLSI guideline EP06-A [18]. A single urine sample that contained elevated concentrations of both biomarkers was mixed with a separate urine sample that contained low concentrations of both biomarkers. These samples were mixed to prepare 9 test samples with IGFBP-7 concentrations between 3.9 and 543.6 ng/ml and TIMP-2 concentrations between 0.9 and 36.0 ng/ml. Each concentration was tested with at least 9 individual NephroCheck Tests. TIMP-2 and IGFBP-7 assay results were compared against their expected values with second and third order polynomial regression models to evaluate linearity.

2.5. Interference studies

The effect of 127 potential interfering substances (Table 1) was evaluated following CLSI guideline EP07-A2 [19]. Each substance was tested at multiple therapeutic or clinically relevant concentrations of the interferent. At each test concentration, the substance was added to a human urine pool that contained approximately 3.5–3.9 ng/ml TIMP-2 and 80.8–92.0 ng/ml IGFBP-7. This urine test sample and a corresponding control urine sample devoid of the potential interferents were then tested with at least 32 individual Nephrocheck Tests. Any substance that exhibited more than a 10% bias in AKIRisk Score results between test and control samples or consistently gave an "invalid" message on the instrument display was considered to interfere with test results.

In addition to the above testing, albumin and bilirubin (conjugated) levels were also measured in urine samples collected from 388 critically ill ICU adult subjects at risk for AKI. These samples were previously collected as part of the Topaz multicenter study as described above [11]. Data from this analysis were used to understand the prevalence of potential albumin and bilirubin (conjugated) interferences. These measurements were conducted by an independent reference lab (ARUP Laboratories, Salt Lake City, UT) using Roche ALBT2 (albumin) and TBILI (total bilirubin) assays.

2.6. Sample stability studies

The stability of the AKIRisk Score with respect to sample storage was evaluated with 126 urine samples collected from critically ill ICU adult subjects at risk for AKI. These samples were collected as part of the

Table 1
Endogenous and exogenous substances tested for interference with the AKIRisk Score.

Dextran 40	Ethacrynic acid	Pancuronium
Pentastarch	Ethanol	Pantoprazole (protonix)
Hetastarch	Fenoldopam	Phenobarbital
Visipaque (iodixanol)	Fentanyl	Phenylephrine
Omniscan (gadodiamide)	Fluconazole	Pravastatin
Omnipaque (iohexol)	Fluvastatin	Prednisone (prednisolone)
Magnevist	Furosemide	Propofol
(Gadopentate dimeglumine)	Gentamicin	Ranitidine
Optiray (ioversol)	Glucose	Riboflavin
Acetaminophen	Hemoglobin	Rocuronium
Acetone	Heparin	Theophylline
Acetylcysteine albumin	Hydralazine	Tobramycin
Aspirin	Hydrochlorothiazide	Torsemide
Acyclovir	Hydrocodone	Urobilinogen
Albuterol	Hydrocortisone	Valproic acid (valproate)
Amiodarone	Ibuprofen	Vancomycin
Ammonia	Insulin	Vasopressin
Amoxicillin	Ketorolac	Vecuronium
Amphotericin	Lansoprazole	Warfarin (coumadin)
Ascorbic acid	Linezolid	Cystatin C
Atorvastatin	Lisinopril	Interleukin-18 (IL-18)
Bicarbonates bilirubin (conjugated)	Lorazepam	Kidney injury molecule 1
Bumetanide	Low molecular weight heparin	Liver type fatty acid binding protein
Caffeine	Mannitol	N-acetyl-β-D-glucosaminidase
Caspofungin	Metformin	Neutrophil gelatinase associated lipocalin (NGAL)
Cefepime	Methylene blue	Pi-glutathione s-transferase
Ceftriaxone	Metolazone	Calcium
Cephalexin	Metoprolol	Chloride
Ciprofloxacin	Metronidazole	Creatinine
Clopidogrel	Midazolam	Magnesium
Dexmedetomidine	Morphine	Phosphate
Diltiazem	Moxifloxacin myoglobin	Potassium
Dopamine	Nitroglycerin	Sodium
Doripenem	Norepinephrine	Sulfate
Epinephrine	Omeprazole	Urea
	Ondanesteron	Uric Acid

Opal multicenter study and followed the Helsinki Declaration of human rights [12]. The study was approved and participants provided written informed consent. Each sample was tested in duplicate within 2 h of collection time to allow for transport of the sample to the laboratory. Separate aliquots of each of these samples were then tested in duplicate after storage at the following conditions: 6 ± 1 h at ambient lab temperatures and 24 ± 4 h at 2–8 °C. A weighted Deming regression analysis was used to compare AKIRisk Score results from each of these storage conditions with the corresponding data generated within 2 h of sample collection.

The stability of the AKIRisk Score with respect to urine centrifugation temperature was evaluated with 127 remnant (otherwise medical waste) urine samples collected from ICU patients. An aliquot of each sample was tested in duplicate after centrifugation in a refrigerated centrifuge set to 4 °C. A separate aliquot of each sample was also tested in duplicate after centrifugation at ambient lab temperatures. A weighted Deming regression analysis was used to compare AKIRisk Score results obtained after room temperature centrifugation with the corresponding results obtained after refrigerated centrifugation.

3. Results

3.1. Precision

Testing of 1123 clinical samples collected from critically ill ICU patients at risk for AKI across multiple laboratories yielded the precision profile for the AKIRisk Score results illustrated in Fig. 1. This precision profile exhibits an average CV of 11% across all samples. Near the recommended AKIRisk Score cutoff of 0.3, the AKIRisk Score result exhibits approximately 10% CV. The imprecision of the AKIRisk Score result increases near the bottom of the reportable range, with approximately 15% CV near the minimum reportable AKIRisk Score result of 0.04.

3.2. Analytical sensitivity

The analytical sensitivity characteristics of the NephroCheck Test are presented in Table 2. The AKIRisk Score exhibited a LoQ value of 0.002, well below its reportable range of 0.04 to 10.0 and recommended clinical cutoff value of 0.3.

3.3. Linearity

The NephroCheck Test exhibited linear TIMP-2 and IGFBP-7 assay results as shown in Table 3. Best fit second and third order non-linear regression analyses of these data produced statistically insignificant ($p > 0.05$) nonlinear regression coefficients indicating that the TIMP-2 and IGFBP-7 assay results are linear. The data in Table 3 demonstrate linearity between 0.9 and 36.0 ng/ml for TIMP-2 and 3.9 and 543.6 ng/ml for IGFBP-7. This corresponds to AKIRisk Score values ranging from 0.003 to 19.6. Additionally, observed TIMP-2 and IGFBP-7 assay results were within 10% of their expected values for all test samples, further demonstrating the linearity of the these results.

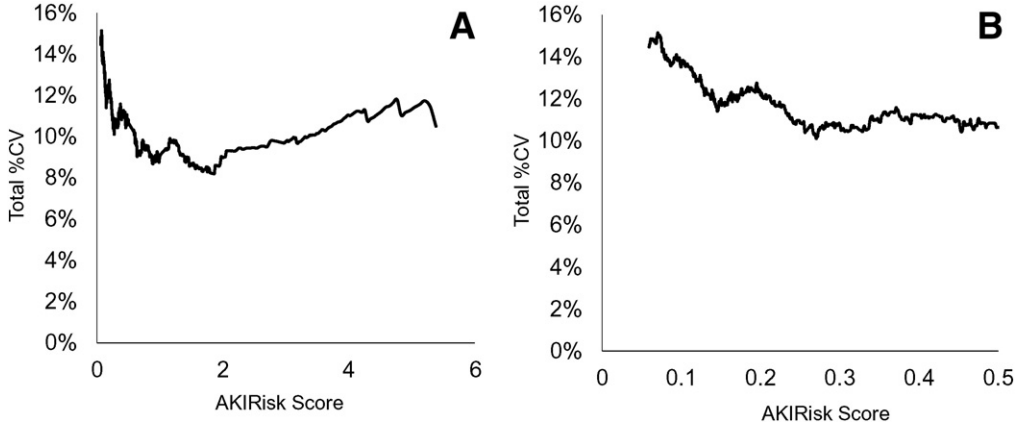


Fig. 1. Precision profile of the AKIRisk Score based on repeated measurements of 1123 clinical specimens at three different laboratories. (A) The Total CV is shown as function of the AKIRisk Score across the reportable range of 0.04-10.00 and (B) near the recommended cutoff value of 0.30.

Table 2
Limit-of-blank (LoB), limit-of-detection (LoD), and limit-of-quantitation (LoQ) of the NephroCheck Test's TIMP-2, IGFBP-7, and AKIRisk Score results.

Result	LoB	LoD	LoQ
TIMP-2	0.5 ng/ml	0.7 ng/ml	0.7 ng/ml
IGFBP-7	0.4 ng/ml	2.8 ng/ml	2.8 ng/ml
AKIRisk Score	0.0002	0.002	0.002

3.4. Interference

The 127 substances listed in Table 1 were evaluated for interference. Albumin, bilirubin (conjugated), and methylene blue were the only substances that interfered with test results. These substances impacted AKIRisk Score results at concentrations exceeding 1250 mg/L, 72 mg/L, and 0.49 mg/L, respectively. No other substances exhibited significant interference.

Elevated urine concentrations of albumin, bilirubin (conjugated), and methylene blue were further tested to characterize how these substances interfere with test results. The outcome of this additional testing is presented in Table 4. Albumin concentrations listed in Table 4 exceeding 1250 mg/L (i.e. ≥2500 mg/L) increased AKIRisk Score results by 15% to 30%. Albumin concentrations of 30,000 mg/L lead to an "invalid" message on the meter rather than a quantitative AKIRisk Score result. Conjugated bilirubin concentrations listed in Table 4 greater than 72 mg/L decreased IGFBP-7, TIMP-2, and AKIRisk Score results by 10% to 20%. Like albumin, elevated methylene blue concentrations listed in Table 4 exceeding 0.49 mg/L lead to an "invalid" message on the meter rather than a quantitative AKIRisk Score result.

To understand the prevalence of the albumin and bilirubin (conjugated) interferences, we analyzed urine samples collected from 388 patients at risk for AKI. These patients suffered from conditions associated with elevated urine albumin, including patients with chronic kidney disease (8%), diabetes (29%), and hypertension (67%). Additionally, this population included patients who suffered from disease conditions associated with elevated bilirubin (conjugated) levels such as liver disease (7%), hepatic failure (2.1%), and cirrhosis (5%). Study results reveal that fewer than 2% of these urine samples had albumin levels that exceeded the concentrations observed to cause interference, and none of these urine samples had bilirubin (conjugated) concentrations observed to cause interference.

3.5. Sample stability

AKIRisk Score results were stable at the ambient and refrigerated sample storage conditions examined. As illustrated in Fig. 2A and B, AKIRisk Score results from 124 critically ill ICU patients obtained after 6 h ambient sample storage and 24 h refrigerated sample storage were equivalent to the corresponding results obtained shortly after sample collection (i.e. within 2 h of sample collection). Regression analyses of the plotted data indicate that AKIRisk Score results change by 1.3% on average after 6 h ambient storage and 2.9% on average after 24 h refrigerated storage. Additionally, the intercept and slope values from all regression analyses were not statistically significant ($p > 0.05$ for intercept and slope values based on a null hypotheses slope = 1 and intercept = 0).

AKIRisk Score results were also stable with respect to centrifugation temperature. The instructions provided with the NephroCheck Test instruct users to centrifuge urine samples in a refrigerated centrifuge prior to testing [14]. Our results indicate that samples are stable at ambient centrifugation temperatures. As illustrated in Fig. 2C, AKIRisk Score results from 127 ICU patient samples obtained after ambient centrifugation were equivalent to the corresponding results obtained after centrifugation under refrigerated conditions. The regression analysis of the data shown in Fig. 2C indicate that AKIRisk Score results differ by 1.3% on average between ambient and refrigerated centrifugation procedures. Additionally, the intercept and slope values from this regression analysis were not statistically significant ($p > 0.05$ for intercept and slope values based on a null hypotheses of intercept = 0 or slope = 1).

4. Discussion

The Nephrocheck Test provides AKIRisk Score results that are analytically accurate and reproducible. The precision study analyzed clinical specimens from 1123 critically ill patients at risk for AKI and demonstrated good reproducibility of the AKIRisk Score result. The total imprecision (CV), accounting for within-run, run-to-run, site-to-site, instrument-to-instrument, and operator-to-operator sources of variability, is 10% to 15% across the AKIRisk Score's reportable range of 0.04 to 10.0. The total imprecision of the AKIRisk Score at the recommended cutoff of 0.3 was 10%.

Analytical sensitivity and linearity study results indicate that the AKIRisk Score has good analytical sensitivity and provides quantitatively accurate results throughout its reportable range. The LoQ of the AKIRisk Score is 20-fold lower than its minimum reportable value of 0.04, demonstrating that has sufficient analytical sensitivity to support its reportable range of 0.04 to 10.0. Furthermore, TIMP-2 and IGFBP-7 results are linear and therefore possess good internal and relative accuracy. Although the AKIRisk Score itself is not linear since it is the multiplicative product of two linear results, it exhibits good internal and relative accuracy given the linearity of its constituent TIMP-2 and IGFBP-7 results.

Interference studies examined the effect of 127 potentially interfering substances relevant to patients at risk for AKI. Only three of these substances exhibited interference. Albumin, bilirubin (conjugated), and methylene blue interfered with test results at the concentrations listed in Table 4 exceeding 1250 mg/L, 72 mg/L, and 0.49 mg/L, respectively. The interferences observed with bilirubin (conjugated) and methylene blue may be caused by interference between the optical properties of these materials and the fluorescent reagents contained

Table 3
Linearity and relative accuracy of the NephroCheck Test's TIMP-2 and IGFBP-7 assays.

IGFBP-7				TIMP-2			
Expected conc. (ng/ml)	Observed conc. (ng/ml)	% recovery	Polynomial regression coefficients p-values	Expected conc. (ng/ml)	Observed conc. (ng/ml)	% recovery	Polynomial regression coefficients p-values
3.87	3.87	100.0%	Second order (×2) p = 0.17	0.94	0.94	100%	Second order (×2) p = 0.54
78.87	71.34	90.5%	Third order (×2) p = 0.25	5.59	5.32	95%	Third order (×2) p = 0.18
146.71	138.82	94.6%	Third order (×3) p = 0.35	10.31	9.69	94%	Third order (×3) p = 0.14
213.17	206.29	96.8%		14.68	14.07	96%	
276.26	273.76	99.1%		18.97	18.45	97%	
350.74	341.23	97.3%		23.17	22.83	99%	
408.73	408.7	100.0%		27.7	27.21	98%	
474.48	476.17	100.4%		31.39	31.58	101%	
543.64	543.64	100.0%		35.96	35.96	100%	

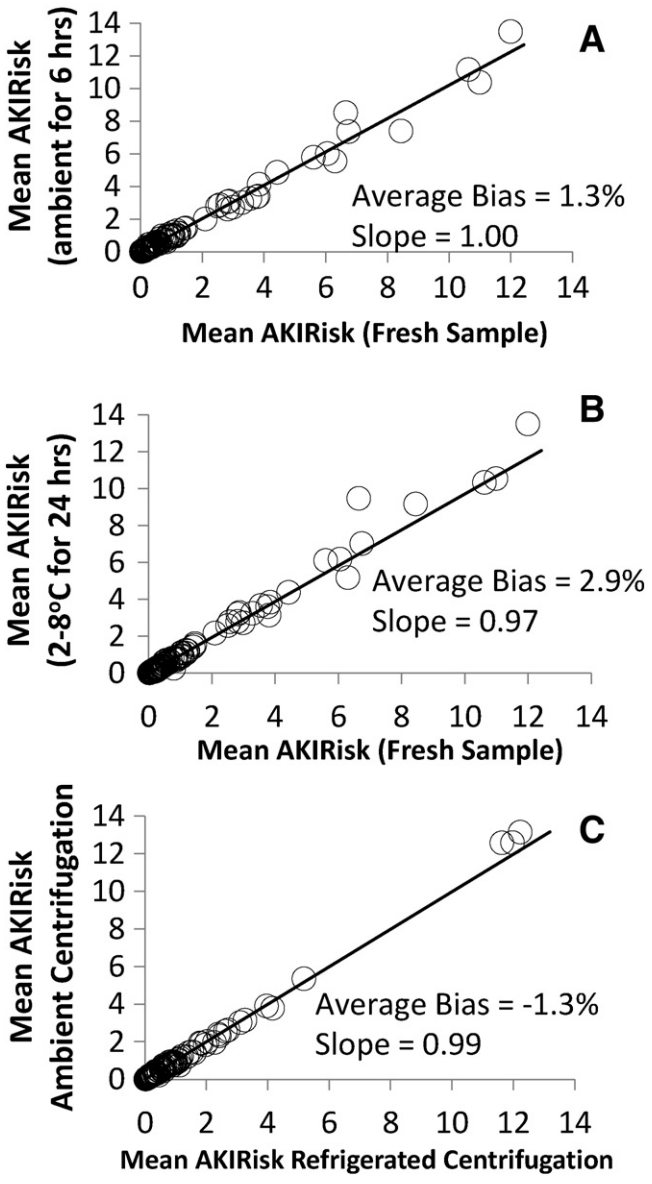
Table 4
Characterization of Albumin, Bilirubin (conjugated) and Methylene Blue Interference.

Albumin conc. (mg/L)	% recovery IGFBP-7 conc.	% recovery TIMP-2 conc.	% recovery AKIRisk conc.
1250	102.1	100.7	103.1
2500	117.5	108.3	128.6
5000	115.9	103.4	119.8
15,000	125.9	103.1	129.7
30,000	No result[a]	No result[a]	No result[a]

Bilirubin (conjugated) conc. (mg/L)	% recovery IGFBP-7 conc.	% recovery TIMP-2 Conc.	% recovery AKIRisk
72	99.1	101.9	101.1
145	89.5	88.7	79.1
217	84.5	88.1	74.3
289	86.5	91.2	79.1

Methylene blue conc. (mg/L)	% recovery IGFBP-7 conc.	% recovery TIMP-2 conc.	% recovery AKIRisk
0.49	100.0	98.3	98.2
1.95	No result[a]	No result[a]	No result[a]
2.93	No result[a]	No result[a]	No result[a]

[a] When no result is reported, the test instrument displayed an "invalid" message rather than a quantitative test result.



within the TIMP-2 and IGFBP-7 immunoassays. In contrast, the interference observed with albumin may be caused by albumin's impact on urine viscosity. It has been reported that high concentrations of albumin increases urine viscosity [20]. This increased viscosity may slow sample flow through the test's lateral flow membrane and thereby impact test results.

Methylene blue is a substance approved for intravenous use to treat methemoglobinopathies [21] and functions to reduce methemoglobin back to hemoglobin. It is also used off label for imaging procedures [22] and to treat low systemic resistance and increased cardiac output following cardiac surgery [23]. As methylene blue causes urine to turn blue, a visual inspection of the urine sample for a blue color would be an indicator of this potential interferent [24]. We found that the test system was able to detect methylene blue interference. When methylene blue interfered with results, the test system displayed an "invalid" message on the meter and no quantitative test result was reported. Since the presence of methylene blue in urine is visually obvious and the instrument displays an "invalid" message when methylene blue interference is present, we conclude that methylene blue interference will have little clinical impact.

Unlike methylene blue, both albumin and bilirubin (conjugated) are naturally occurring substances in the body. Normal levels of albumin in urine range from 0 to 80 mg/L [20]. Urine albumin levels may be elevated above this range in clinical populations with chronic kidney disease, diabetes, glomerular disease, and hypertension [25]. While conjugated bilirubin is normally not present in urine [26]; urine bilirubin (conjugated) may be elevated in patients with liver damage or dysfunction [27]. Unlike methylene blue, the presence of albumin or bilirubin may not be obvious in urine samples because these substances do not necessarily change the visual appearance of urine and the test does not necessarily report an "invalid" AKIRisk Score result when albumin or bilirubin (conjugated) interferences are present.

Although albumin and bilirubin (conjugated) can interfere with test results, we found that concentrations that would cause interference are seldom present in patients at risk for AKI. We analyzed urine albumin and bilirubin (conjugated) concentrations in samples collected from 388 patients at risk for AKI. Fewer than 2% of patients had albumin

Fig. 2(A) A comparison of the AKIRisk score results from samples obtained within 2 hours of sample collection (fresh samples; x-axis) and the same samples obtained after 6 hours of storage at ambient temperatures (y-axis). (B) A comparison of the AKIRisk score results from samples obtained within 2 hours of sample collection (fresh samples; x-axis) and the same samples obtained after 24 hours storage at 2-8°C (y-axis). (C) A comparison of the AKIRisk score results from 127 patient samples centrifuged at ambient (x-axis) and refrigerated (y-axis) temperatures.

concentrations that would interfere with test results. No patients exhibited elevated bilirubin (conjugated) concentrations.

The sample stability study demonstrates that AKIRisk Score results exhibit good sample stability. Urine samples may be stored for up to 6 h at room temperature and 24 h at refrigerated conditions. This stability is sufficient to support the intended use of the NephroCheck Test. In particular, the test is intended to be used for risk assessment of moderate or severe kidney injury within 12 h of sample collection and patient assessment [14]. Based on this intended use, clinicians have a limited time period (i.e. no longer than 12 h from sample collection) to act upon the AKIRisk Score results [14]. Therefore, although our results indicate that urine samples may be stored for 6 or more hours, clinical laboratories should avoid prolonged urine sample storage and test urine samples as soon as possible to maximize the clinical risk assessment value of the AKIRisk Score result and its impact on patient care.

Additional stability studies show that the AKIRisk Score result is also stable at ambient centrifugation temperatures. Centrifugation of urine samples prior to analysis is generally recommended to remove any sediment, casts, or other solid materials in the urine that may interfere with test results [24]. The test's instructions-for-use state that urine samples should be refrigerated during centrifugation [14]. However, our results indicate that refrigerated and ambient centrifugation yield equivalent AKIRisk Score test results. Therefore, refrigerated centrifugation of urine samples prior to analysis is unnecessary, and urine samples may be centrifuged at ambient temperatures with no negative impact on test results.

In conclusion, the NephroCheck Test is the first FDA cleared test to assess risk of developing acute kidney injury. Our studies demonstrate that the results of this test have good precision, acceptable analytical sensitivity, minimal analytical interference, and are robust to sample handling and processing conditions.

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